PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07H 21/00, A61K 31/70, C12N 15/11 — A1

(11) International Publication Number: **WO 00/06588**

(43) International Publication Date: 10 February 2000 (10.02.00)

(21) International Application Number: PCT/US99/17100

(22) International Filing Date: 27 July 1999 (27.07.99)

(30) Priority Data:
60/094,370 27 July 1998 (27.07.98) US

(71) Applicants: UNIVERSITY OF IOWA RESEARCH FOUNDATION [US/US]; 214 Technology Innovation Center, Oakdale Research Campus, Iowa City, IA 52242 (US). CPG IMMUNOPHARMACEUTICALS, INC. [US/US]; Suite 120, 55 William Street, Wellesley, MA 02481 (US).

(72) Inventor: KRIEG, Arthur, M.; 890 Park Place, Iowa City, IA 52246 (US).

(74) Agent: LOCKHART, Helen, C.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

Date of publication of the amended claims:
6 April 2000 (06.04.00)

(54) Title: STEREOISOMERS OF CpG OLIGONUCLEOTIDES AND RELATED METHODS

(57) Abstract

The interactions of nucleic acids with proteins can be selective for the R stereoisomer, the S stereoisomer, or can be stereoindependent. The present invention demonstrates that the S stereoisomer of CpG containing DNA is active in mediating the immune stimulatory effects of CpG DNA. The invention provides methods of use of a pure stereoisomer or of DNA enriched for this form for clinical applications for CpG DNA, such as vaccine adjuvants, immune activators for the prevention or treatment of retroviral, viral, parasitic or fungal diseases, or cancer immunotherapy, immunotherapy of allergic and asthmatic diseases, etc. The invention also provides methods of use for R stereoisomer DNA to oppose the immune stimulatory effects of CpG DNA. Such R stereoisomers are useful in the treatment of diseases such as Sepsis syndrome, intestinal inflammatory diseases, psoriasis, gingivitis, systemic lupus erythematosus and other autoimmune diseases.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

AMENDED CLAIMS
[received by the International Bureau on 24 January 2000 (24.01.00);
Original claims 1, 29, 35-39, 46, 47, 50-52, 54-56, 60, 74, 80 and 82 amended;
remaining claims unchanged (13 pages)]

1. A composition comprising:

an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

2. The composition of claim 1, wherein X_1X_2 are nucleotides selected from the group consisting of: TpA, ApA, ApC, ApG, and GpG.

3. The composition of claim 1, wherein X_3X_4 are nucleotides selected from the group consisting of: TpT, TpA, TpG, ApA, ApG, GpA, and CpA.

4. The composition of claim 1, wherein X_1X_2 are nucleotides selected from the group consisting of: TpT, TpG, ApT, GpC, CpC, CpT, TpC, GpT and CpG; X_3 is a nucleotide selected from the group consisting of A and T and X_4 is a nucleotide, but wherein when X_1X_2 is TpC, GpT, or CpG, X_3X_4 is not TpC, ApT or ApC.

5. The composition of claim 1, wherein the immunostimulatory nucleic acid is double stranded.

6. The composition of claim 1, wherein less than all of the nucleotides have a backbone modification.

7. The composition of claim 1, wherein less than all of the chiral centers have S chirality.

8. The composition of claim 1, wherein at least 50% of the nucleotides have backbone modifications.

9. The composition of claim 1, wherein at least 75% of the nucleotides have backbone modifications.

10. The composition of claim 1, wherein at least 90% of the nucleotides have backbone modifications.

11. The composition of claim 1, wherein at least 60% of the chiral centers have S chirality.

12. The composition of claim 1, wherein at least 75% of the chiral centers have S chirality.

13. The composition of claim 1, wherein at least 90% of the chiral centers have S chirality.

14. The composition of claim 1, wherein the immunostimulatory nucleic acid is single stranded.

15. The composition of claim 1, wherein the immunostimulatory nucleic acid has a sequence including at least the following formula:

$$5' \text{ TCNTX}_1\text{X}_2\text{CGX}_3\text{X}_4 \text{ 3}'$$

wherein N is a nucleic acid sequence composed of from about 0-25 nucleotides.

16. The composition of claim 1, wherein the composition includes immunostimulatory nucleic acids having identical sequences.

17. The composition of claim 1, wherein the composition includes immunostimulatory nucleic acids having at least two different sequences.

18. The composition of claim 17, wherein the at least two sequences include a B-cell activating sequence and an NK cell activating sequence.

19. The composition of claim 1, wherein the nucleic acid has less than or equal to 100 nucleotides.

20. The composition of claim 1, wherein the nucleic acid has between 8 and 40 nucleotides.

21. The composition of claim 1, further comprising an antigen.

22. The composition of claim 1, further comprising a cytokine.

23. The composition of claim 22, wherein the cytokine is selected from the group consisting of GM-CSF, IL-4, IL-18, IFNα, TNFα, Flt3 ligand, and IL-3.

24. The composition of claim 1, further comprising an antiviral.

25. The composition of claim 1, further comprising an antibacterial.

26. The composition of claim 1, further comprising a non-nucleic acid adjuvant.

27. The composition of claim 1, wherein the composition is formulated as a sustained release device.

28. The composition of claim 1, wherein the CpG formula is a palindrome.

29. A composition comprising:

a double stranded immunostimulatory nucleic acid having a sequence on one strand including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

30. The composition of claim 29, wherein X_1X_2 are nucleotides selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG.

31. The composition of claim 29, X_3X_4 are nucleotides selected from the group consisting of:TpT, CpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

32. The composition of claim 29, wherein X_1X_2 are nucleotides selected from the group consisting of: GpT, GpG, GpA and ApA; and X_3X_4 are nucleotides selected from the group consisting of: TpT, CpT and ApT.

33. The composition of claim 29, wherein less than all of the nucleotides have a backbone modification.

34. The composition of claim 29, wherein less than all of the chiral centers have S chirality.

35. A method of inducing an antigen-specific immune response in a subject comprising:

administering to a subject an antigen and an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality, in an amount effective to induce an antigen- specific immune response.

36. A method for redirecting a subject's immune response from a Th2 to a Th1 comprising:

administering to a subject an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality, in an amount effective to redirecting the subject's immune response from a Th2 to a Th1.

37. A method for treating asthma in a subject, comprising:

administering to an asthmatic subject an effective amount for treating asthma in the subject of an immunostimulatory nucleic acid having a sequence including at least the following formula: 5' X_1 X_2CGX_3 X_4 3'

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

38. A method for desensitizing a subject against the occurrence of an allergic reaction in response to contact with an allergen, comprising administering to a subject an immunostimulatory nucleic acid having a sequence including at least the following formula:

5' X_1 X_2CGX_3 X_4 3'

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

39. A method for activating an immune cell, comprising:

isolating an immune cell from a subject,

contacting the immune cell with an effective amount to activate the immune cell of an immunostimulatory nucleic acid having a sequence including at least the following formula:

5' X_1 X_2CGX_3 X_4 3'

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality, and

readministering the activated immune cells to the subject.

40. The method of claim 39, wherein the immune cell is a lymphocyte.

41. The method of claim 40, further comprising contacting the immune cell with an antigen.

42. The method of claim 41, wherein the antigen is selected from the group consisting of: a tumor antigen, a viral antigen, a bacterial antigen, and a parasitic antigen.

43. The method of claim 39, wherein the immune cell is a dendritic cell.

44. The method of claim 43, wherein the dendritic cell expresses a cancer antigen.

45. The method of claim 44, wherein the dendritic cell is exposed to the cancer antigen *ex vivo*.

46. A method for activating a dendritic cell, comprising:

contacting a dendritic cell with an effective amount to activate a dendritic cell of an immunostimulatory nucleic acid having a sequence including at least the following formula:

5' X_1 X_2CGX_3 X_4 3'

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

47. A method for treating a cancer, comprising:

administering to a subject having a cancer an effective amount for treating the cancer of an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

48. The method of claim 47, wherein the method is method for increasing the responsiveness of a cancer cell to a cancer therapy and wherein the immunostimulatory nucleic acid is administered in conjunction with an anti-cancer therapy.

49. The method of claim 48, wherein the anti-cancer therapy is an antibody.

50. A method for enhancing recovery of bone marrow in a cancer therapy subject, comprising:

administering to a subject undergoing or having undergone cancer therapy which damages the bone marrow an effective amount for enhancing the recovery of bone marrow of an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

51. In a method for stimulating an immune response in a subject having a cancer, the method of the type involving antigen dependent cellular cytotoxicity (ADCC), the improvement comprising:

administering to the subject an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

52. A method for inducing cytokine production in a subject comprising

administering to the subject an effective amount to induce a cytokine in the subject of an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

53. The method of claim 52, wherein the cytokine is selected from the group consisting of Il-6, Il-12, IL18 TNF, IFN α and IFN-γ.

54. A method of stimulating natural killer cell lytic activity comprising

exposing a natural killer cell to an immunostimulatory nucleic acid to stimulate natural killer cell lytic activity, the immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality.

55. A method of inducing a Th1-type immune response in a subject, comprising:

administering to the subject in order to induce a Th1 immune response a combination of adjuvants, wherein the combination of adjuvants includes at least one immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality, and at least one non-nucleic acid adjuvant, and wherein the combination of adjuvants is administered in an effective amount for inducing a Th1-type immune response.

56. A method for inducing a mucosal immune response, comprising:

administering to a mucosal surface of a subject an effective amount for inducing a mucosal immune response of an immunostimulatory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have S chirality, and

exposing the subject to an antigen to induce the mucosal immune response.

57. The method of claim 56, wherein the antigen is not encoded in a nucleic acid vector.

58. The method of claim 56, wherein the antigen is encoded by a nucleic acid vector.

59. The method of claim 56, wherein the mucosal surface is selected from the group consisting of an oral, nasal, rectal, vaginal, and ocular surface.

60. A composition comprising:

an immunoinhibitory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2 CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have R chirality.

61. The composition of claim 60, wherein X_1X_2 are nucleotides selected from the group consisting of: TpA, ApA, ApC, ApG, and GpG.

62. The composition of claim 60, X_3X_4 are nucleotides selected from the group consisting of: TpT, CpT, TpA, TpG, ApA, ApG, GpA, and CpA.

63. The composition of claim 60, wherein X_1X_2 are nucleotides selected from the group consisting of: TpT, TpG, ApT, GpC, CpC, CpT, TpC, GpT and CpG; X_3 is a nucleotide selected from the group consisting of A and T and X_4 is a nucleotide, but wherein when X_1X_2 is TpC, GpT, or CpG, X_3X_4 is not TpC, ApT or ApC.

64. The composition of claim 60, wherein the immunoinhibitory nucleic acid is double stranded.

65. The composition of claim 60, wherein less than all of the nucleotides have a backbone modification.

66. The composition of claim 60, wherein less than all of the chiral centers have R chirality.

67. The composition of claim 60, wherein at least 50% of the nucleotides have backbone modifications.

68. The composition of claim 60, wherein at least 75% of the nucleotides have backbone modifications.

69. The composition of claim 60, wherein at least 90% of the nucleotides have backbone modifications.

70. The composition of claim 60, wherein at least 60% of the chiral centers have R chirality.

71. The composition of claim 60, wherein at least 75% of the chiral centers have R chirality.

72. The composition of claim 60, wherein at least 90% of the chiral centers have R chirality.

73. The composition of claim 60, wherein the immunoinhibitory nucleic acid is single stranded.

74. A composition comprising:

a double stranded immunoinhibitory nucleic acid having a sequence on one strand including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have R chirality.

75. The composition of claim 74, wherein X_1X_2 are nucleotides selected from the group consisting of: GpT, GpG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT, and TpG.

76. The composition of claim 74, X_3X_4 are nucleotides selected from the group consisting of: TpT, CpT, ApT, TpG, ApG, CpG, TpC, ApC, CpC, TpA, ApA, and CpA.

77. The composition of claim 74, wherein X_1X_2 are nucleotides selected from the group consisting of: GpT, GpG, GpA and ApA; and X_3X_4 are nucleotides selected from the group consisting of: TpT, CpT and ApT.

78. The composition of claim 74, wherein less than all of the nucleotides have a backbone modification.

79. The composition of claim 74, wherein less than all of the chiral centers have R chirality.

80. A method of preventing an immune response in a subject comprising:

administering to a subject having an excessive immune response an immunoinhibitory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center and wherein a plurality of the chiral centers have R chirality, in an amount effective to prevent an immune response.

81. The method of claim 80, wherein the subject having an excessive immune response is a subject who has received an immune stimulating compound.

82. A method for treating a subject comprising:

administering to a subject having or at risk of having an inflammatory disease an immunoinhibitory nucleic acid having a sequence including at least the following formula:

$$5' X_1 X_2CGX_3 X_4 3'$$

wherein C is unmethylated, wherein X_1, X_2, X_3 and X_4 are nucleotides, wherein at least two nucleotides have a phosphate backbone modification forming a chiral center

and wherein a plurality of the chiral centers have R chirality, in an amount effective to prevent induction of an immune response.

83. The method of claim 82, wherein the inflammatory disease is selected form the group consisting of inflammatory bowel disease, autoimmune disease, gingivitis, psoriasis, and sepsis.

84. The method of claim 1, wherein the nucleic acid has a sequence including at least the formula $GTCGTX_4$.

85. A method for inducing antigen non-specific innate immune activation and broad spectrum resistance to infectious challenge